Persistence of Virus in Urine as Factor in Spread of Infectious Hepatitis in Dogs.* (18749)

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Although infectious hepatitis virus is not airborne(1), the disease is highly infectious and spreads easily. Susceptible dogs placed with infected ones readily became infected, but when separated by only 6 inches no infection occurred. Further study revealed that ingestion of saliva from infected dogs during the febrile period caused disease. Dogs apparently recovered from the disease still infected those placed in contact with them, although tests then of their saliva, blood and feces revealed no virus. Studies were, therefore, made on other body secretions to determine the source of this persistent virus. Attention focused on the urine and the finding was made that animals eliminated infective virus in the urine long after apparent recovery from the disease. As has been reported for a number of other viruses, its presence in urine might be expected during viremia, but the exceptionally long persistence in the presence of circulating neutralizing antibodies appears an unusual finding for a virus. Although this method of spread is used by leptospira(2,3), it suggests a new concept of virus dissemination.

Methods. Litters of puppies, approximately 1 to 4 months old, were placed in an isolation room so that they would not be exposed to any unplanned infection. At the time of inoculation, the dogs were distributed to individual kennel units separated by a distance of more than 6 inches. Since all dogs in a litter respond in a similar manner to an inoculation of the virus of infectious hepatitis, at least one animal in each litter was shown to be susceptible before others were used for test purposes. Dogs were each inoculated intravenously into the radial vein with 1 cc of a 10% suspension in saline of spleen from an infected dog. After inoculation, temperatures were taken twice each day and a leukocyte count was made daily. Blood was removed from the jugular vein before inoculation and afterwards at daily intervals for 21 days. During this same period, urine was collected daily and thereafter at weekly intervals. All specimens were frozen immediately with a mixture of dry ice and alcohol and placed under dry ice refrigeration. In a test for virus, a specimen was thawed and 1 cc inoculated intravenously into a test dog. Presence of virus was determined by fever, leukopenia and other general signs of illness, finding gross changes in the liver and edema of the gall bladder on autopsy and intranuclear inclusions in histopathological studies. In some instances, dogs were held and given a spleen suspension that had been shown to produce infection in a control animal. Failure to show signs of illness indicated that the test animal had been immunized by virus in the specimen.

Results. As can be seen in Table I, virus was present in 5 tests of blood and in 4 tests of urine made on inoculated dogs during the period of clinical illness. As shown by tests on 5 separate dogs, virus disappeared from the blood at the end of fever and leucopenia but was present in the urine of dogs No. 61 when tested 10 days after inoculation; No. 6, 20 days afterwards; No. 22, 21 days; No. 40, 7, 18 and 41 days; and No. 15, 65, 76, 80, 137 and 161 days afterwards.

Inasmuch as virus was found in urine after recovery, pathological studies were made on the kidneys. Kidneys of puppies examined during the febrile stage showed slight degenerative changes, particularly in the tubules,

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TABLE I. Presence and Persistence of Virus in Blood and Urine of Dogs Inoculated Intravenously with Virus of Infectious Hepatitis.

Dog No.	Clinical signs* Fever Onset	Fever End	Leucopenia Onset	Leucopenia End	Test for virus Days after inoculation	Blood	Urine
6†	2	6	2	7	20	—	+
15	2	7	3	7	65	N.T.	+
					76	N.T.	+
					80	N.T.	+
					137	N.T.	+
					161	—	+
22†	2	8	3	8	21	—	+
40	2	6	3	6	2	+	—
					3	+	+
					4	+	+
					6	+	+
					7	—	+
					18	—	+
					41	N.T.	+
55	0	0	2	3	3	+	+
61	2	5	2	4	10	—	+

* Indicates days after inoculation.
† Serum contained neutralizing antibodies. N.T. = not tested.

with some protein casts. Inclusion bodies were found in the glomerular endothelium (Fig. 1). In kidneys of puppies that recovered from the disease, widespread but irregularly shaped areas of round cell infiltration were seen, usually in the region of the corticomedullary junction. Adjacent to the patches of cellular infiltration, mononuclear cells were

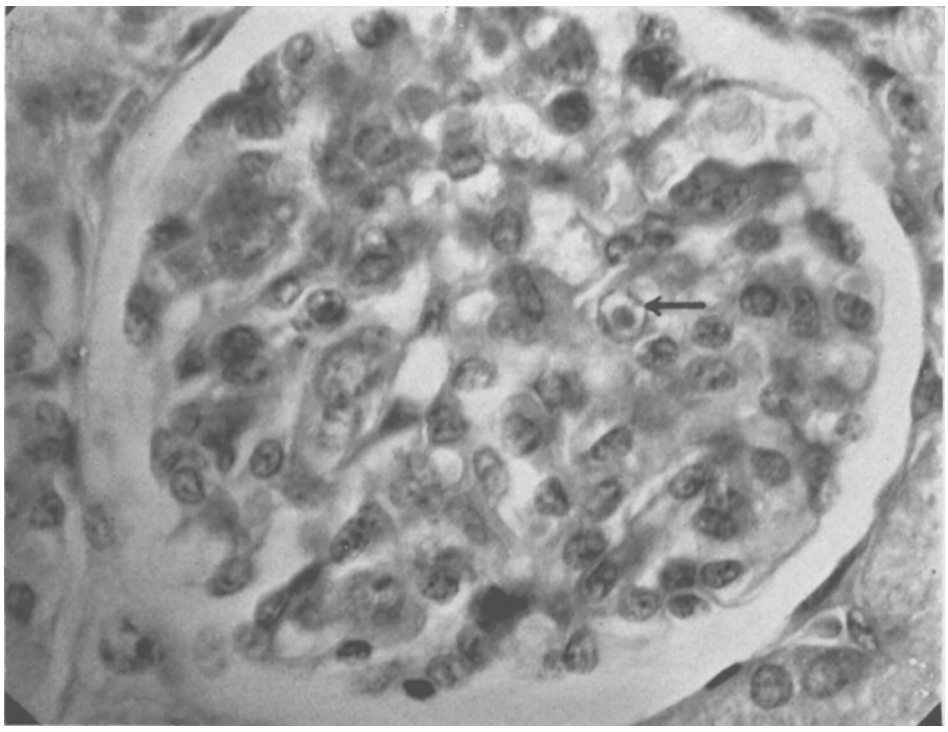

FIG. 1. Kidney from dog showing signs of illness. Note intranuclear inclusion bodies characteristic of the virus of infectious hepatitis.

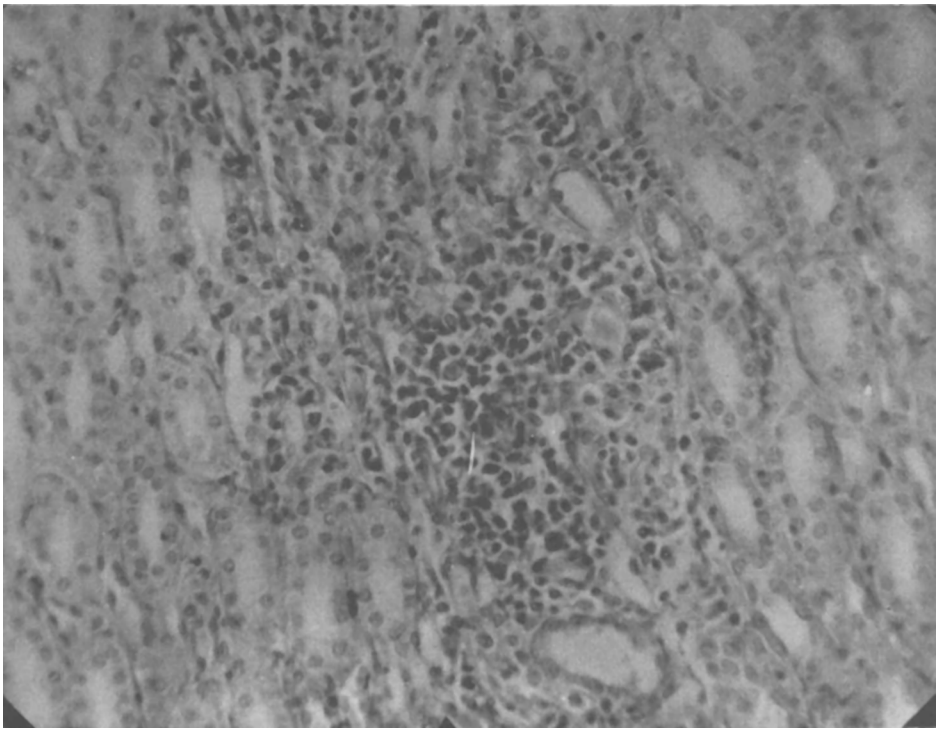

FIG. 2. Kidney from dog after recovery from infectious hepatitis. Note cellular infiltration.

seen between tubules normally occupied by peritubular capillaries (Fig. 2). No evidence of collagen formation or fibrosis was found.

Conclusions. The virus of infectious hepatitis of dogs has been recovered from the urine at intervals of time extending from 3 days after inoculation to at least 161 days afterwards. Inasmuch as blood, saliva, nasal washings and feces from apparently recovered dogs did not contain virus, it appears that infected urine is important in the spread of the disease. Finding a focal interstitial nephritis indicated that the source of virus eliminated in the urine is the kidney.

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Cutaneous Sarcoma-like Lesions of the Horse Caused by the Agent of Bovine Papilloma.* (18750)

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While studying the transmissibility of a cutaneous papilloma of the horse(1) it was found that the horse would respond to the agent of bovine papilloma. The response involved connective tissue rather than epithelial tissue and a sarcoma-like tumor was produced

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